European Journal of Pharmaceutical Sciences 199 (2024) 106809

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Identification of non-adherence to adjuvant letrozole using a population pharmacokinetics approach in hormone receptor-positive breast cancer patients

Alicja Puszkiel [a], Florence Dalenc [b], Naïma Tafzi [c], Pierre Marquet [c], Marc Debled [d], William Jacot [e], Laurence Venat-Bouvet [f], Catherine Ferrer [g], Nadia Levasseur [h], Rodolphe Paulon [i], Jérôme Dauba [j], Alexandre Evrard [e,k], Vincent Mauriès [b], Thomas Filleron [b], Etienne Chatelut [a,b], Fabienne Thomas [a,b,1], Melanie White-Koning [a,1,*]

[a] Cancer Research Center of Toulouse (CRCT), Inserm U1037, Université Paul Sabatier - Toulouse III, 2 Avenue Hubert Curien, Toulouse 31100, France
[b] Institut Claudius Regaud, Institut Universitaire du Cancer de Toulouse – Oncopole, Toulouse, France
[c] Department of Pharmacology, Toxicology and Pharmacovigilance, CHU Limoges, Limoges, France
[d] Department of Medical Oncology, Institut Bergonié, Bordeaux, France
[e] Institut du Cancer de Montpellier, ICM, Université de Montpellier, IRCM, Inserm U1194, Montpellier, France
[f] Department of Medical Oncology, CHU Limoges, Limoges, France
[g] Department of Medical Oncology, CHU Nîmes-Carémeau, Nîmes, France
[h] Department of Medical Oncology, CH Cahors, Cahors, France
[i] Department of Medical Oncology, Centre Hospitalier Intercommunal Castres-Mazamet, Castres, France
[j] Department of Medical Oncology, CH Mont de Marsan, Mont-de-Marsan, France
[k] Laboratoire de Biochimie et Biologie Moléculaire, CHU Nîmes-Carémeau, Nîmes, France

ARTICLE INFO

Keywords:
Letrozole
Pharmacokinetics
Non-linear mixed-effects modelling
CYP2A6
CYP3A4/5
Adherence

ABSTRACT

Background: Letrozole, an aromatase inhibitor metabolised via CYP2A6 and CYP3A4/5 enzymes, is used as adjuvant therapy for women with hormone receptor (HR)-positive early breast cancer. The objective of this study was to quantify the impact of CYP2A6 genotype on letrozole pharmacokinetics (PK), to identify non-adherent patients using a population approach and explore the possibility of a relationship between non-adherence and early relapse.

Methods: Breast cancer patients enrolled in the prospective PHACS study (ClinicalTrials.gov NCT01127295) and treated with adjuvant letrozole 2.5 mg/day were included. Trough letrozole concentrations ($C_{ss,trough}$) were measured every 6 months for 3 years by a validated LC-MS/MS method. Concentration-time data were analysed using non-linear mixed effects modelling. Three methods were evaluated for identification of non-adherent subjects using the base PK model.

Results: 617 patients contributing 2534 plasma concentrations were included and led to a one-compartment PK model with linear absorption and elimination. Model-based methods identified 28 % of patients as non-adherent based on high fluctuations of their $C_{ss,trough}$ compared to 3 % based on patient declarations. The covariate analysis performed in adherent subjects revealed that CYP2A6 intermediate (IM) and slow metabolisers (SM) had 21 % (CI95 % = 12 – 30 %) and 46 % (CI95 % = 41 – 51 %) lower apparent clearance, respectively, compared to normal and ultrarapid metabolisers (NM+UM). Early relapse (19 patients) was not associated with model-estimated, concentration-based or declared adherence in the total population ($p = 0.41$, $p = 0.37$ and $p = 0.45$, respectively).

Conclusions: These findings will help future investigations focusing on the exposure-efficacy relationship for letrozole in adjuvant setting.

* Corresponding author.
E-mail address: melanie.white-koning@univ-tlse3.fr (M. White-Koning).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejps.2024.106809
Received 27 November 2023; Received in revised form 5 April 2024; Accepted 21 May 2024
Available online 22 May 2024

1. Introduction

Letrozole is a non-steroidal third generation aromatase inhibitor used in combination or not with ovarian suppression, for the treatment of women with early stage or advanced hormone receptor (HR)-positive breast cancer (Waks and Winer, 2019,2). Its mechanism of action consists in prevention of hormone-dependent growth of cancer cells by inhibiting aromatase-mediated conversion of androgens to estrogens.

The mean half-life of letrozole is 48 h and steady-state is reached after 2 to 6 weeks of treatment (Highlights of Prescribing Information [Internet], 2024). Its major route of elimination is metabolism into an inactive carbinol metabolite via both CYP2A6 and CYP3A4/5 isoenzymes and excretion in urines mainly as a glucuronide metabolite (Pfister et al., 2001; Desta et al., 2011). The intrinsic clearance of letrozole is twice as high for CYP2A6 as for CYP3A4 (0.48 nl/min/nmol versus 0.24 nl/min/nmol, respectively) (Murai et al., 2009). In addition, CYP2A6 plays a major role in letrozole metabolism at low concentrations (0.5 μmol/L corresponding to 142.6 ng/mL which is close to therapeutic concentrations) whereas at concentrations high above therapeutic values (1426 ng/mL), CYP2A6 is saturated and CYP3A4 takes over the role of primary metabolism. Hence, at therapeutic concentrations, CYP2A6 is the predominant enzyme involved in letrozole metabolism. It has been reported that there is a 10- to 16-fold variation in plasma exposure to letrozole among patients (Desta et al., 2011; Borrie et al., 2018). This high inter-individual variability (IIV) might be related to variable activity of the metabolising enzymes. Indeed, around 50 variant alleles for both *CYP2A6* (Tanner and Tyndale, 2017) and *CYP3A4* gene (Werk and Cascorbi, 2014) have been reported.

The occurrence of adverse events and the extensive duration of adjuvant treatment may impact patient adherence to treatment (defined as less than 80 % of prescribed doses taken Waterhouse et al., 1993; Fisher et al., 1989). Indeed, it has been reported that approximately 10 %, 13 % and 18 % of patients treated with adjuvant aromatase inhibitors are non-adherent during the first, second and third year of the therapy, respectively (Huiart et al., 2011). However, available methods to record adherence (electronic devices, medical and self-reports) are often biased and Pistilli et al. have reported only a moderate agreement between self-reported adherence and actual plasma levels of tamoxifen (Pistilli et al., 2020). Therefore, identification of non-adherent subjects based on several steady-state plasma concentrations might constitute a promising approach to estimate adherence.

This study aimed to: (1) develop a population PK model for letrozole based on longitudinal steady-state data in patients under adjuvant therapy, (2) identify non-adherent patients using the PK model, (3) compare the model-estimated adherence, the declared adherence and the concentration-based adherence and explore the possibility of a relationship with early breast cancer relapse, (4) evaluate the impact of genetic polymorphisms, demographic characteristics and CYP3A4 inhibitors on letrozole PK in the adherent subpopulation as identified by the PK model.

2. Material and methods

2.1. Study population

Data come from a prospective, multicenter, 3-year follow-up study aiming to investigate the relationship between PK, pharmacogenetics (PG) and adverse events of tamoxifen and aromatase inhibitors in adjuvant breast cancer patients (PHACS; ClinicalTrials.gov NCT01127295). The inclusion criteria were: histologically proven primary breast cancer, no metastatic disease at diagnosis, ER–positive and/or progesterone receptor (PR)–positive tumour assessed by locally performed immunohistochemistry. Eligible patients started treatment with tamoxifen (20 mg/day) or one of the aromatase inhibitors (letrozole (2.5 mg/day), anastrozole (1 mg/day), exemestane (25 mg/day)) and were followed-up every 6 months over 3 years. This report focuses on the analysis of all the patients who were treated with letrozole alone at some point during the study including those who started treatment with letrozole at inclusion and those who switched to letrozole during the course of the study after having been treated with tamoxifen or another aromatase inhibitor. During each follow-up visit, data on adverse events, co-medications and declared adherence during the month preceding the visit were collected. Declared adherence was reported by the clinician based on the patient's answers to questions regarding their adherence during the previous 30 days. The ratio of the number of doses taken in the last 30 days to the number of theoretical doses was expressed as a percentage. Patients who had taken less than 80 % of the doses at least at one follow-up visit (where they were treated with letrozole) were considered as non-adherent. Early relapse was defined as recurrent disease (metastases and /or loco-regional recurrence), contralateral breast cancer, or death attributed to breast cancer during the first 3 years of treatment. Only the patients who had at least 3 complete years of follow-up were included in the relapse analysis. All patients provided written informed consent as per the revised Declaration of Helsinki and French regulations.

2.2. Determination of letrozole plasma concentrations

Blood samples for letrozole quantification were collected in 5 mL lithium-heparin tubes at inclusion (pre-treatment) and 24-hours post-dose every 6 months during the follow-up visits. Plasma concentrations of letrozole were quantified by a validated high-performance liquid chromatography-mass spectrometry (LC-MS/MS) method described in detail in **Supplementary Material S1**.

2.3. Genotyping

Blood samples for genotyping were collected in 7 mL EDTA tubes at study inclusion for all the participants. Genotyping for *CYP2A6*1, *2, *9, CYP3A4*22, *1B* and *CYP3A5*3* and CYP2A6 copy number variation (CNV) analysis was performed by IntegraGen SA, Evry, France and is detailed in **Supplementary Material S2**.

Patients were classified into CYP2A6 phenotype as proposed previously (Chenoweth et al., 2013). Normal metabolisers (NM) had no variant alleles (genotype **1/*1*), intermediate metabolisers (IM) had one copy of a decreased function variant allele (genotype **1/*9*) and slow metabolisers (SM) had two copies of a decreased function variant allele or one or two copies of a loss-of-function variant allele (genotypes **9/*9, *1/*2, *2/*2, *2/*9*). Patients with more than 2 copies of the CYP2A6 gene and no variant alleles were classified as ultrarapid metabolisers (UM). Due to the small number of patients in the UM category, NM and UM patients were combined into NM+UM category. Patients with *CYP3A5*1/*1* and **1/*3* genotypes were classified as CYP3A5 expressers whereas patients with **3/*3* genotype as non-expressers.

2.4. Population pharmacokinetic analysis

2.4.1. Model development

The concentration-time data were analysed using nonlinear mixed effects modelling in NONMEM software version 7.4.1 (ICON Development Solutions, Ellicott City, Maryland). Estimation of the parameters was performed using first order conditional estimation with interaction method. One- and two-compartment models with first order absorption and linear elimination were fit to the data. The IIV of the PK parameters was modelled according to an exponential model, i.e. assuming a log-normal distribution of the PK parameters. The inter-occasion variability (IOV) was explored. The proportional and combined error models were tested for the residual variability. The concentrations below lower limit of quantification (LLOQ = 1 ng/mL) were included in the PK model with values fixed to LLOQ/2.

2.4.2. Identification of non-adherence using PK model

Non-adherence to treatment is most often due to missed doses, which could lead to lower bioavailability (F) of the drug. Hence, a possible approach to identify non-adherent subjects could assume that in those subjects, the F is decreased. Another method to identify non-adherence was proposed by Gibiansky et al. (2014) and is based on the individual residual error estimates as detailed below.

Three methods were tested on the base PK model in order to identify the non-adherent patients:

- **Method 1**

Method 1 (M1) assumes decreased bioavailability (F) of letrozole in non-adherent patients. A mixture model was included on F and each patient was assigned to one of two subpopulations: adherent ($F_{adh} = 1$, fixed) or non-adherent ($0 < F_{non\text{-}adh} < 1$) (Garcia-Cremades et al., 2019). Since one patient could be adherent at some visits and non-adherent at others, the inclusion of IOV on $F_{non\text{-}adh}$ was tested (each follow-up visit was considered as a separate occasion).

- **Method 2**

Another approach (M2) was proposed by Gibiansky et al. (2014) and relies on the assumption that non-adherent subjects show high fluctuations of trough concentrations at steady-state ($C_{ss,trough}$) between multiple occasions which is reflected by a high residual error when the model does not include IOV. Therefore, the non-adherent subjects could be identified based on their individual residual error estimates. The inclusion of a random effect $\eta_{i,\sigma}$ on the residual error makes it possible to investigate the distribution of residual error and search for subpopulations. For this method, the residual error was described according to the proportional model and the random effect $\eta_{i,\sigma}$was included on the residual error as follows:

$$C_{obs,ij} = C_{pred,ij} + C_{pred,ij} \cdot \varepsilon_{p,ij} \cdot exp(\eta_{i,\sigma})$$

Where $C_{obs,ij}$and $C_{pred,ij}$ represent the observed and predicted concentration, respectively, for the i^{th} subject and the j^{th} measurement, $\varepsilon_{p,ij}$ is the proportional residual error for the i^{th} subject and j^{th} measurement and $\eta_{i,\sigma}$ is the individual value of the random effect on the residual error for the i^{th} subject. $\eta_{i,\sigma}$ is assumed to follow a normal distribution with mean 0 and variance ω^2_σ. In this manner, the identification of adherent and non-adherent subjects can be performed based on assumption that non-adherent subjects have $\eta_{i,\sigma} > 0$ while adherent subjects have $\eta_{i,\sigma} < 0$ as proposed by Gibiansky et al. (2014). Additionally, to demonstrate the impact of non-adherence on mean estimate of CL/F, a sequential exclusion of patients with the highest $\eta_{i,\sigma}$ values from the model was performed.

- **Method 3**

The third method (M3) is inspired by the previous consideration on M2 and consists in estimation of a coefficient $\theta_{non\text{-}adh}$ on the residual error according to the following equation:

$$C_{obs,ij} = C_{pred,ij} + C_{pred,ij} \cdot \varepsilon_{p,ij} \cdot \theta_{non-adh}$$

A mixture model is used to assign each subject into one of two subpopulations with different values of the coefficient: $\theta_{non\text{-}adh} = 1$ (fixed) for adherent and $\theta_{non\text{-}adh} > 1$ for non-adherent subjects, based on the difference between the observed and the model-predicted concentration (i.e. non-adherent subjects have a higher residual error).

A patient was considered model-based adherent if identified as such by all three methods.

2.4.3. Identification of non-adherence using letrozole concentrations

No plasma concentration threshold has been proposed to identify non-adherent patients for letrozole. However Dragvoll et al. (2022) reported 15 % non-adherence among patients treated with an aromatase inhibitor for HR-positive breast cancer. Hence we identified the letrozole concentration value corresponding to the 15th percentile in our study (48.1 ng/mL) and considered that all patients with mean concentrations (over all visits) lower than this value could be considered non-adherent based on their concentrations.

2.4.4. Covariate analysis

The covariate analysis was performed in adherent patients identified in the PK analysis using the base model. Visual exploration of the relationship between covariates and individual PK parameters was performed to search for possible associations. The following covariates were evaluated: body weight (BW), age, CYP2A6 phenotype, *CYP3A4*22* genotype (wild-type versus **22* carriers), *CYP3A4*1B* genotype (wild-type versus **1B* carriers), *CYP3A5*3* genotype (expressers versus non-expressers) and concomitant use of CYP3A4 inhibitors. When CYP2A6 phenotype was included in the model, a separate coefficient was estimated for SM, IM and missing phenotype (5.5 % of patients), with NM+UM phenotype as the reference. The missing *CYP3A4*22, *1B* and *CYP3A5*3* genotypes represented less than 2.0 % of the dataset and therefore were imputed with the most frequent category (wild-type for *CYP3A4*22* and **1B* and non-expressors for *CYP3A5*3* genotype). The continuous covariates were included in the model according to a linear function and were scaled to the population median whereas a coefficient was estimated for each group for categorical covariates (the reference group was the most frequent category). The methods for covariates selection are detailed in **Supplementary Material S3**.

2.4.5. Model evaluation and validation

During model development, a drop of at least 3.84 points in objective function value (OFV, $p < 0.05$, 1 df, $\chi 2$ test) between nested models was considered statistically significant. The selection of the model was also based on goodness-of-fit plots, precision (relative standard error, RSE), stability and plausibility of the estimates. Goodness-of-fit plots included a prediction-corrected visual predictive check (pcVPC) based on 1000 simulations of the original dataset. A bootstrap analysis with resampling ($n = 500$) was performed with the final model.

2.5. Statistical analysis

For descriptive analyses, qualitative variables were expressed as number (%) and quantitative variables as median [range]. Statistical analyses were performed in R (version 4.1.2) coupled with RStudio (version 2022.07.1 + 554) and Stata (version 15.1). The comparison of baseline demographic and biological characteristics as well as of the number of comedications between non-adherent and adherent patients (as estimated by population PK model) was performed using the Wilcoxon unpaired test for continuous variables and χ^2 or Fisher's exact test for categorical variables. The agreement between model-estimated, concentration-based and declared adherence was calculated as well as the associated kappa-statistic and Cramér's V coefficient. The association between adherence and early relapse was tested using χ^2 or Fisher's exact test.

3. Results

3.1. Patients and data

Table 1 summarizes the demographic and genetic characteristics of 617 patients included in the analysis. A total of 2534 letrozole concentrations were analysed. Of those, 46 (1.8 %) concentrations were below LLOQ. The median sampling time was 24.2 h (interquartile range [23.2 – 25.2]) after dose. All the studied genotypes were in Hardy-Weinberg equilibrium after Bonferroni correction for multiple testing. Of the 617 patients included in the analysis, 390 started treatment with

Table 1
Baseline characteristics and concomitant treatment during study of the total study population ($n = 617$).

Characteristic	median [range] or number (%)
Age at inclusion (years)	61 [40 – 84]
Body weight (kg)	66 [37 – 122]
Hormonal status	
Pre-menopause	67 (10.9 %)
Post-menopause	540 (87.5 %)
Menopause under substitutive hormone treatment	3 (0.5 %)
Unknown	7 (1.1 %)
CYP2A6 phenotype	
Normal or ultrarapid (NM + UM)	458 (78.6 %)
Intermediate (IM)	52 (8.9 %)
Slow (SM)	73 (12.5 %)
Missing	34
*CYP3A4*22* genotype	
Wild-type (**1/*1*)	561 (92.4 %)
Heterozygous mutant (**1/*22*)	45 (7.4 %)
Homozygous mutant (**22/*22*)	1 (0.2 %)
Missing	10
*CYP3A4*1B* genotype	
Wild type (**1/*1*)	565 (92.3 %)
Heterozygous mutant (**1/*1B*)	46 (7.5 %)
Homozygous mutant ((**1B/*1B*)	1 (0.2 %)
Missing	5
*CYP3A5*3* genotype	
Wild type (**1/*1*)	5 (0.8 %)
Heterozygous mutant (**1/*3*)	93 (15.3 %)
Homozygous mutant ((**3/*3*)	509 (83.9 %)
Missing	10
Co-medications	Number of occasions (%)
Weak[a] CYP3A4 inhibitors	68 (2.7 %)
Moderate/potent[b] CYP3A4 inhibitors	57 (2.2 %)

[a] weak CYP3A4 inhibitors: esomeprazole;fluvoxamine;fluoxetine, efavirenz;.
[b] moderate/potent CYP3A4 inhibitors: amiodarone, ciprofloxacin, clarithromycin, diltiazem, erythromycin, fluconazole, indinavir, ketoconazole, verapamil.

letrozole at study inclusion and stayed on it throughout the study, while 227 patients switched to or from letrozole during the course of the study (tamoxifen or another aromatase inhibitor being the alternative treatment).

3.2. Population pharmacokinetic analysis

The concentration-time data were described by a one-compartment model with linear absorption and elimination. The parameters of the model were: apparent clearance (CL/F), apparent volume of distribution (V/F) and first order absorption rate constant (k_a). A two-compartment model was also tested but the PK parameters (peripheral volume of distribution V_p/F and inter-compartmental clearance Q/F) and associated IIV were estimated with high imprecision without any significant benefit in terms of residual variability. The IIV was included only on CL/F and was fixed to 0 for V/F and k_a as it could not be reliably estimated. The inclusion of IOV on CL/F did not improve the model. The residual variability was described by a combined (base model) or proportional (M1, M2 and M3) error model.

3.2.1. Identification of non-adherent patients

The performance of three different approaches to identify non-adherent subjects was investigated on the base model and the mean parameter estimates of these methods are presented in Table 2.

Inclusion of a mixture model on F (M1) resulted in a drop in OFV of 59 points ($p < 0.00001$). The estimated $F_{non\text{-}adh}$ was 0.006 (RSE = 2 %) and fraction of the non-adherent subpopulation was 2 %. The individual fit plots showed that only the patients with concentrations near LLOQ throughout all the visits were identified as non-adherent (Fig. 1, Patient 2).

Table 2
Mean parameter estimates obtained using three approaches to identify non-adherent patients in the base PK model ($n = 617$ patients).

Parameter	Mean estimate (RSE%) [shrinkage%]			
	Base model	M1[a]	M2[b]	M3[c]
k_a (h^{-1})	2.16 (43)	2.21 (40)	2.13 (59)	1.91 (39)
V/F (L)	186 (12)	197 (15)	181 (15)	172 (12)
CL/F (L/h)	1.27 (2)	1.25 (2)	1.24 (2)	1.27 (2)
F_{adh}	–	1 FIX	–	–
$F_{non\text{-}adh}$	–	0.006 (2)	–	–
$\theta_{non\text{-}adh}$	–	–	–	2.59 (5)
Estimated fraction of adherent population	–	0.98 (1)	–	0.74 (6)
Proportion of adherent patients (%) (obtained by post-hoc analysis)	–	98.0	72.0	84.0
Inter-individual variability on CL/F (CV%)	47.6 (5) [5]	40.7 (4) [4]	45.9 (7) [2]	49.6 (8) [4]
Inter-individual variability on proportional residual error (CV %)	–	–	41.5 (7) [19]	–
Proportional error (CV%)	18.4 (8) [11]	24.1 (3) [10]	19.7 (6) [7]	16.6 (9) [16]
Additive error (ng/mL)	10.9 (10)	–	–	–

CL/F apparent clearance, *CV* coefficient of variation, F_{adh} relative bioavailability of the adherent population, $F_{non\text{-}adh}$, relative bioavailability of the non-adherent population, k_a first order absorption rate constant, $\theta_{non\text{-}adh}$ coefficient for non-adherent patients estimated on the residual error, *RSE* relative standard error, *V/F* apparent volume of distribution.
[a] M1 – Method based on inclusion of a mixture model on F.
[b] M2 – Method based on estimation of a random effect $\eta_{i,\sigma}$ on residual error.
[c] M3 – Method based on inclusion of a mixture model on $\theta_{non\text{-}adh}$.

M2 consisted in separation of patients into two subpopulations based on $\eta_{i,\sigma}$. When the non-adherent subjects were considered as those with $\eta_{i,\sigma} > 0$, 28 % of patients were identified as non-adherent (Table 2). Fig. 2 demonstrates the impact of a sequential exclusion of patients with the highest $\eta_{i,\sigma}$ values on mean estimate of CL/F. This approach assumes that non-adherent patients have low plasma concentrations which may lead to an artificially high estimate of CL/F. Exclusion of 30 % of patients resulted in a drop of 7.0 % in mean CL/F estimate compared to the entire dataset (1.16 L/h versus 1.27 L/h). Further exclusion of patients did not alter mean CL/F estimate suggesting that all patients with non-adherence patterns were excluded.

In M3, a mixture model was used to assign subjects into two populations based on $\theta_{non\text{-}adh}$ estimate. The probability of being in the non-adherent population was estimated by the model as being 26 %.

In conclusion, M2 and M3 based on the residual error identified a higher percentage of non-adherent patients than M1. Indeed, M2 and M3 assigned all patients with high fluctuations of $C_{ss,trough}$ to non-adherent population whereas M1 considered as non-adherent only the patients with very low concentrations (< LLOQ) throughout all the study. Overall, the three methods identified 175 (28 %) patients as non-adherent (**Table S1**).

3.2.2. Covariate analysis

Since PK analysis in a population with non-adherence patterns could lead to biased estimates, the covariate analysis was performed on the adherent subpopulation (442 patients contributing 1705 concentrations). The characteristics of the population included in the covariate analysis are presented in **Table S2**. The comparison between adherent and non-adherent patients showed no statistically significant differences for any of the covariates except for the BW ($p = 0.045$) and intake of CYP3A4 inhibitors ($p = 0.004$). The plots of the individual CL/F

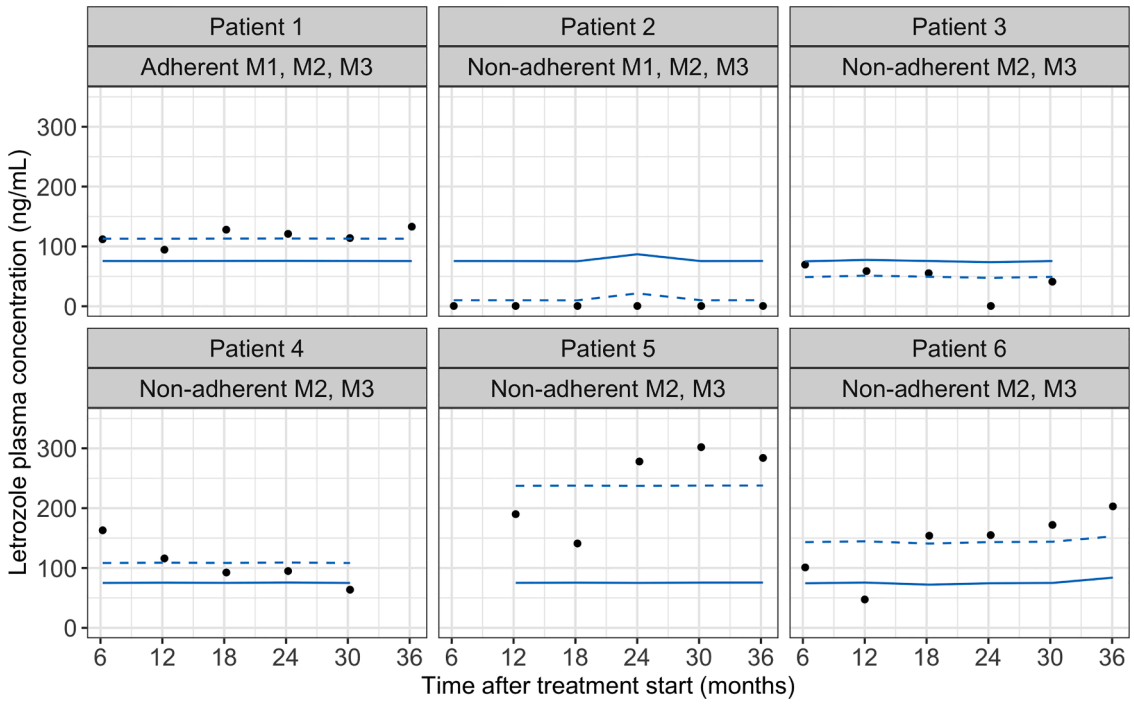

Fig. 1. Individual pharmacokinetic profiles of 6 representative patients and the performance of M1, M2 and M3 to identify non-adherence. Points represent observations, dotted line represents individual predictions (IPRED) and solid line represents population prediction (PRED) from the base model.

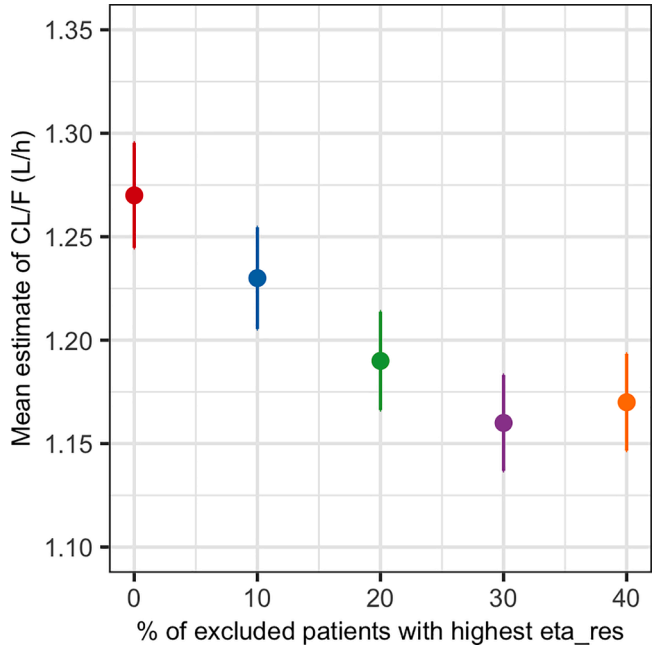

Fig. 2. Variation in CL/F following sequential exclusion of patients with the highest $\eta_{i,\sigma}$ (eta_res) as estimated by M2. Dots represent the point estimate and bars represent the associated standard error.

obtained from the base model versus CYP2A6 phenotype, *CYP3A4*22, *1B* and *CYP3A5*3* genotypes as well as BW and age are presented on Fig. 3. In the univariable analysis, CYP2A6 phenotype ($p < 0.00001$) was the only covariate significantly associated with CL/F. Adding CYP2A6 phenotype led to a decrease in IIV on CL/F from 42.4 % in the base model to 35.7 % in the final model. According to the estimates of the final model, CYP2A6 IM and SM patients had 21 % and 46 % lower CL/F, respectively, than NM+UM patients. Finally, *CYP3A4*22, CYP3A4*1B* and *CYP3A5*3* genotypes and concomitant CYP3A4 inhibitors were not significant covariates on CL/F ($p = 0.20$, $p = 0.80$, and $p = 0.27$ and $p = 0.37$, respectively).

The estimates of the final model in the adherent subpopulation and the results of the bootstrap analysis are presented in Table 3. The goodness-of-fit plots and pcVPC are presented in **Fig. S1** and **Fig. S2**, respectively. IPRED and PRED versus observed concentrations show that the model describes the data well and the residual diagnostics CWRES versus PRED and time do not show a significant misspecification of the model. The pcVPC stratified on CYP2A6 phenotype showed good agreement between the model-predicted median, 5th and 95th percentiles of the concentrations and the observed data.

3.3. Comparison between model-estimated, concentration-based and declared adherence

Of 571 patients for whom adherence data was available, 17 (3 %) declared non-adherence to the clinician in at least one follow-up visit whereas the model-estimated non-adherence was 28 %. Table 4 presents the comparison between model-estimated and declared adherence and also the median concentrations (based on mean concentrations over all visits) for each category. Among 442 patients identified as adherent in the PK analysis, the information concerning declared adherence was available for 406 patients. Of the latter, 4 patients declared non-adherence. This discrepancy may be explained by misunderstanding of the questionnaire. Among 554 patients who self-declared as adherent, 152 (27 % out of 554) were identified as non-adherent by the PK model suggesting that those patients do not admit their non-adherence. Among 17 patients who declared non-adherence, 13 (76 %) were identified as non-adherent by the PK model. The agreement between declared and model-estimated adherence was 73 % (CI95 %: 71 – 75 %), which is significantly greater than the (by chance) expected agreement of 70 %, but only slightly so. Furthermore, the corresponding kappa-statistic was 0.09 and Cramér's coefficient was 0.18, indicating only slight agreement between adherence assessment methods. Agreement between declared and concentration-based adherence was 84 % (CI95 %: 80 – 87 %), only slightly greater than the expected chance agreement of 83 %, with a

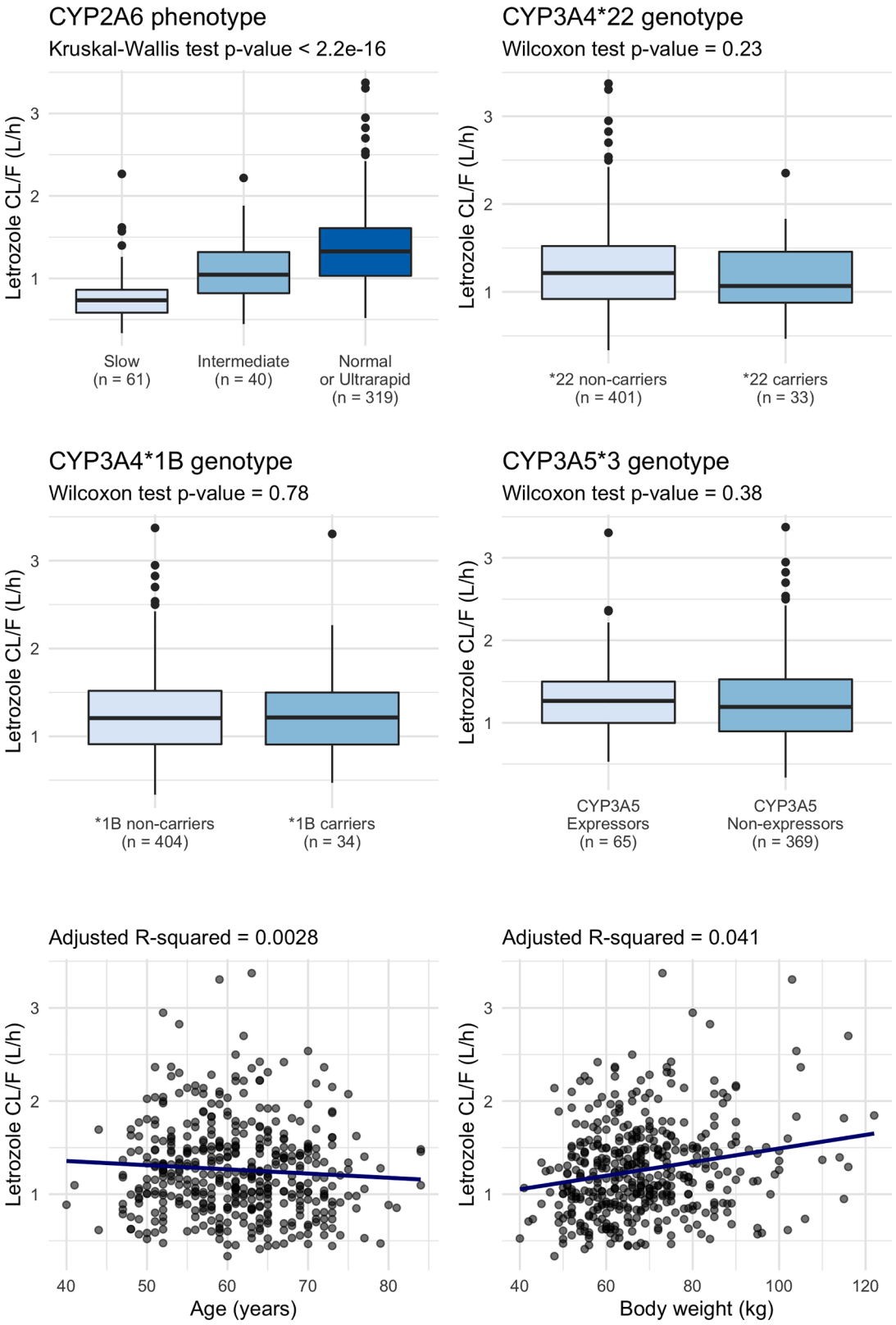

Fig. 3. Individual apparent clearance (CL/F) from the base model for adherent patients (n = 442) according to CYP2A6 phenotype, CYP3A4*22, CYP3A4*1B and CYP3A5*3 genotypes, age and body weight.

kappa-statistic of 0.07 and a Cramér's coefficient of 0.10. Agreement between model-estimated and concentration-based adherence was 73 % (CI95 %: 69 – 77 %), but this was significantly greater than the expected chance agreement of 65 %, with a kappa-statistic of 0.23 and a Cramér's coefficient of 0.25, indicating better agreement between these two adherence assessment methods.

Table 3
Estimates of the base and final PK model in adherent patients ($n = 442$) and results of non-parametric bootstrap analysis of the final model ($n = 500$).

Parameter	Mean estimate (RSE%) [shrinkage]		Bootstrap mean (CI95 %)
	Base model	Final model	
k_a (h^{-1})	2.18 (55)	2.12 (53)	2.24 (1.11 – 8.35)
V/F (L)	209 (10)	207 (10)	208 (167 – 249)
CL/F (L/h)	1.17 (2)	1.31 (2)	1.30 (1.26 – 1.35)
Effect of CYP2A6 IM on CL/F	–	0.79 (6)	0.79 (0.69 – 0.89)
Effect of CYP2A6 SM on CL/F	–	0.54 (5)	0.55 (0.49 – 0.60)
Effect of missing CYP2A6 on CL/F	–	0.85 (8)	0.85 (0.72 – 0.99)
IIV on CL/F (CV%)	42.4 (3) [2]	35.7 (4) [3]	35.6 (32.8 – 38.6)
Proportional error (CV%)	14.3 (4) [13]	14.3 (4) [13]	14.2 (13.7 – 14.7)

The final equation to predict the individual CL/F_i was as follows: $CL/F_i = 1.31\ (L/h) \cdot 0.79^{IM} \cdot 0.54^{SM} \cdot 0.85^{missing} \cdot \exp(\eta_{CL/Fi})$ where *IM, SM* and *missing* equal 1 if the patient has CYP2A6 IM, SM or missing phenotype, respectively, and 0 otherwise and $\eta_{CL/Fi}$ is the estimated IIV on CL/F for the i^{th} subject.
CI confidence interval, *CL/F* apparent clearance, *CV* coefficient of variation, *CYP2A6 IM* CYP2A6 intermediate metabolizer phenotype, *CYP2A6 SM* CYP2A6 slow metabolizer phenotype, *IIV* inter-individual variability, k_a first order absorption rate constant, *RSE* relative standard error, *V/F* apparent volume of distribution.

Table 4
Comparison between model-estimated and declared adherence.

Declared	Model-estimated		Total
	Adherent	Non-adherent	
Adherent	73 % ($n = 402$)	27 % ($n = 152$)	97 % ($n = 554$)
	82.8 [63.1–114.0]*	60.9 [46.0–80.4]*	75.1 [57.9–105.8]*
Non-adherent	24 % ($n = 4$)	76 % ($n = 13$)	3 % ($n = 17$)
	83.5 [42.1–99.3]*	51.3 [27.9–80.2]*	60.9 [27.9–84.2]*
Total	71 % ($n = 406$)	29 % ($n = 165$)	100 % ($n = 571$)
	82.9 [63.1–114.0]*	60.8 [45.7–80.2]**	74.7 [57.0–104.4]*

* Median of (mean concentrations over all visits for each patient) [interquartile range].

3.4. Exploration of relationship between adherence and early relapse

In the population of patients who had 3 full years of follow-up ($n = 435$), early relapse was reported in 19 patients within those first 3 years of treatment. In this population (including patients who switched treatment), early relapse was not significantly associated with model-estimated adherence ($p = 0.41$), self-declared adherence ($p = 0.45$) or concentration-based adherence ($p = 0.37$). The test was also performed in the subpopulation of patients who stayed on letrozole throughout the first 3 years of treatment ($n = 275$ patients) and had non-missing adherence data ($n = 255$). In patients who had not switched treatments, early relapse was not significantly associated with model-estimated, concentration-based or declared adherence ($p = 0.69$, $p = 0.45$ and $p = 0.59$, respectively). There was no significant difference ($p = 0.84$) between the median concentration of the mean over all visits in the 19 patients who had an early relapse (median = 75.7 ng/mL, IQR = [57.9–105.4]) and the median concentration in patients who did not relapse (median = 75.8 ng/mL, IQR = [57.9–104.7]).

4. Discussion

This study presents a population PK analysis of letrozole as adjuvant endocrine therapy in breast cancer patients (the first such model in European patients) based on data from a prospective longitudinal study.

In our study, the sparse concentration-time data for letrozole were described by a one-compartment model with linear absorption and elimination. The mean estimates of CL/F (1.31 L/h in CYP2A6 NM+UM patients, CI95 % = 1.26 – 1.36) and V/*F* (207 L, CI95 % = 166 – 248) are consistent with the values from a previously published non-compartmental analysis in 24 breast cancer patients (median CL/F at steady-state = 1.20 L/h and median V/*F* = 183 L) (Pfister et al., 2001).

The individual PK profiles in some patients showed high fluctuations of $C_{ss,trough}$ throughout the study whereas some patients had < LLOQ concentrations at all visits suggesting non-adherence. Three different methods were used to identify the non-adherent subpopulation using the base PK model. In M1, the fraction of non-adherent patients estimated by the mixture model was 2 %. However, this approach only assigned to the non-adherent subpopulation patients with very low concentrations (< LLOQ) at all visits. Both M2 and M3 assumed that non-adherent patients were those with high fluctuations of $C_{ss,trough}$ (i.e. high residual error) between multiple occasions and both resulted in the assignment of 28 % of patients as non-adherent.

We considered all three methods since one subject identified as non-adherent by M1 was adherent according to M2 and M3. However, this patient had $C_{ss,trough}$ near LLOQ throughout all study visits and since no fluctuations were observed, M2 did not identify this subject as non-adherent. Overall, we identified 28 % of patients as non-adherent based on all three methods. This model-estimated proportion is more consistent with the non-adherence reported in recent studies in breast cancer cohorts treated with adjuvant tamoxifen or aromatase inhibitor (between 13.1 and 26.8 % in the study by Dragvoll et al., 2022 and 28 % in the study by Hershman et al., 2021) than the percentage of declared non-adherence we found (3 %). In addition to our model-estimated non-adherence that reflects high fluctuations of $C_{ss,trough}$ in line with occasional adherence, we also considered poor adherence leading to under-exposure by taking into account concentration-based adherence. However, the large interindividual variability of letrozole PK means that the identification of non-adherence using a concentration threshold is difficult to implement and subject to caution. Indeed, since adherence is often under-reported by patients themselves, a combination of PK-based methods with conventional approaches might bring more insight into the true adherence during adjuvant endocrine therapy.

High fluctuations of $C_{ss,trough}$ may be related not only to adherence to treatment but also to intake of CYP inhibitors or inducers not reported in the study. Indeed, in our study, a more frequent use of CYP3A4 inhibitors was observed in the non-adherent compared to the adherent subpopulation. This could explain the higher $C_{ss,trough}$ fluctuations observed in those patients. Nevertheless, CYP3A4 inhibitors did not have a significant impact on CL/F in the entire population of this study as evaluated in the PK model (data not shown). Finally, analytical variability is unlikely to be a confounding factor in our analysis since for a given patient, all the samples were quantified by LC-MS/MS on the same day.

In our study, there did not seem to be any association between model-estimated, concentration-based or declared non-adherent patients and early relapse (within the first 3 years) but the number of events was very small (19 patients) given the short time period since initiation of adjuvant therapy. Moreover, our population includes patients treated with letrozole for different time spans since some switched to or from another endocrine therapy during the course of the study. In a recent study, non-adherence to adjuvant tamoxifen has been associated with a higher risk of distant recurrence or death at 3 years after tamoxifen serum assessment (Pistilli et al., 2020). Therefore, our results need to be interpreted with caution, as it may be possible that non-adherence affects long-term relapse. In addition, declared non-adherence might not always be the best marker of exposure to the drug not only because of high discordance between the real and declared adherence. Indeed, for drugs with long elimination half-life, such as letrozole, even if a patient omits several doses in a month, plasma exposure can still be sufficient for pharmacological efficacy. Therefore,

the studies on relationship between non-adherence and cancer relapse should also consider drug plasma exposure.

The covariate analysis revealed that CYP2A6 phenotype was the only covariate significantly associated with CL/F. In particular, IM and SM patients had 21 % and 46 %, respectively, lower CL/F than NM+UM patients. This is consistent with previous reports showing that impaired CYP2A6 activity due to genetic polymorphisms resulted in increased plasma exposure to letrozole, compared to patients with normal CYP2A6 activity (Desta et al., 2011; Borrie et al., 2018). Finally, CYP2A6 phenotype explained 15.8 % of the IIV in letrozole CL/F in our analysis, consistently with Borrie et al. (17.0 %) (Borrie et al., 2018) whereas Desta et al. reported a slightly higher percentage (23 %) probably due to a more extensive *CYP2A6* genotyping in their study (Desta et al., 2011).

In our analysis, *CYP3A4*22, *1B* and *CYP3A5*3* genetic polymorphisms were not correlated with letrozole CL/F. Desta et al. (2011) found no impact of *CYP3A5×3* on letrozole concentrations while the effect of *CYP3A4*22 and *1B* genetic polymorphisms has never been investigated in breast cancer patients. In addition, in our analysis, concomitant CYP3A4 inhibitors did not have a significant impact on letrozole CL/F but the number of occasions corresponding to concomitant use of a CYP3A4 inhibitor was low (4.9 % of the total dataset). Nevertheless, it has been demonstrated in vitro that CYP3A4 is a low-affinity component involved in the formation of the carbinol metabolite of letrozole compared to CYP2A6 (Murai et al., 2009). Therefore, our study confirms that CYP2A6 is the most important enzyme involved in the inactivation of letrozole in vivo and that CYP3A4 plays a minor role.

Previous studies have reported that patients who were older and had lower BMI had higher plasma letrozole concentrations (Desta et al., 2011; Borrie et al., 2018). In the present analysis, letrozole CL/F was not significantly correlated with age nor BW.

5. Conclusion

The longitudinal design of this study allowed for the quantification of non-adherence based on plasma steady-state concentrations of letrozole. In addition, we confirm that CYP2A6 plays an important role in the elimination of letrozole. The results of this study show that as many as 28 % of patients are non-adherent to adjuvant letrozole treatment as evaluated by their plasma concentrations. We did not find any correlation between model-estimated, concentration-based or declared non-adherence and early cancer relapse. However, the relationship between non-adherence to adjuvant hormone therapy and long-term efficacy should be further investigated and drug plasma exposure should be considered.

Declarations

Funding

The PHACS study was supported by a grant from the French Ministry of Health (PHRC 2009 #09–18–005). Alicja Puszkiel received a grant from French National Institute of Health and Medical Research (Inserm).

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author after authorisation from the principal investigator upon reasonable request.

CRediT authorship contribution statement

Alicja Puszkiel: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Project administration, Validation, Visualization. **Florence Dalenc:** Conceptualization, Investigation, Project administration, Writing – review & editing, Funding acquisition, Supervision. **Naïma Tafzi:** Data curation, Resources, Writing – review & editing. **Pierre Marquet:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Marc Debled:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **William Jacot:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Laurence Venat-Bouvet:** Data curation, Resources, Writing – review & editing. **Catherine Ferrer:** Data curation, Resources, Writing – review & editing. **Nadia Levasseur:** Data curation, Resources, Writing – review & editing. **Rodolphe Paulon:** Data curation, Resources, Writing – review & editing. **Jérôme Dauba:** Data curation, Resources, Writing – review & editing. **Alexandre Evrard:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Vincent Mauriès:** Data curation, Resources, Writing – review & editing, Project administration. **Thomas Filleron:** Conceptualization, Data curation, Methodology, Project administration, Writing – review & editing. **Etienne Chatelut:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Fabienne Thomas:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Melanie White-Koning:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests to declare that are relevant to the content of this article.

Data availability

The data are not openly available due to reasons of sensitivity but are available from the corresponding author after authorisation from the principal investigator upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2024.106809.

References

Borrie, A.E., Rose, R.V., Choi, Y.-H., Perera, F.E., Read, N., Sexton, T., et al., 2018. Letrozole concentration is associated with CYP2A6 variation but not with arthralgia in patients with breast cancer. Breast Cancer Res. Treat. 172, 371–379. Springer US.

Chenoweth, M.J., O'Loughlin, J., Sylvestre, M.P., Tyndale, R.F., 2013. CYP2A6 slow nicotine metabolism is associated with increased quitting by adolescent smokers. Pharmacog. Genom. 23, 232. NIH Public Access.

Desta, Z., Kreutz, Y., Nguyen, A.T., Li, L., Skaar, T., Kamdem, L.K., et al., 2011. Plasma letrozole concentrations in postmenopausal women with breast cancer are associated with CYP2A6 genetic variants, body mass index, and age. Clin. Pharmacol. Ther. 90, 693–700.

Dragvoll, I., Bofin, A.M., Søiland, H., Taraldsen, G., Engstrøm, M.J., 2022. Predictors of adherence and the role of primary non-adherence in antihormonal treatment of breast cancer. BMC Cancer 22, 1–12. BioMed Central Ltd.

Fisher, B., Costantino, J., Redmond, C., Poisson, R., Bowman, D., Couture, J., et al., 1989. A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor–positive tumors. N. Engl. J. Med. 320, 479–484. Massachusetts Medical Society.

Garcia-Cremades, M., Vučićević, L., Hendrix, C., Jarlsberg, L., Grant, R., Celum, C., et al. Individual level data meta-analysis from HIV pre-exposure prophylaxis (PrEP) clinical trials. PAGE 28 (2019) Abstr 9196 [www.page-meeting.org/?abstract=9196]HIGHLIGHTS OF PRESCRIBING INFORMATION [Internet]. Accessed March 2024. Available from: www.fda.gov/medwatch.

Gibiansky, L., Gibiansky, E., Cosson, V., Frey, N., Stark, F.S., 2014. Methods to detect non-compliance and reduce its impact on population PK parameter estimates. J. Pharmacokinet. Pharmacodyn. 41, 279–289. Springer US.

Hershman, D.L., Neugut, A.I., Moseley, A., Arnold, K.B., Gralow, J.R., Henry, N.L., et al., 2021. Patient-reported outcomes and long-term nonadherence to aromatase inhibitors. JNCI J. Natl. Cancer Inst. Oxford Acad. 113, 989–996.
Highlights of Prescribing Information [Internet]. 2024 Available from: www.fda.gov/medwatch.
Huiart, L., Dell'Aniello, S., Suissa, S., 2011. Use of tamoxifen and aromatase inhibitors in a large population-based cohort of women with breast cancer. Br. J. Cancer 104, 1558. Nature Publishing Group.
Murai, K., Yamazaki, H., Nakagawa, K., Kawai, R., Kamataki, T., 2009. Deactivation of anti-cancer drug letrozole to a carbinol metabolite by polymorphic cytochrome P450 2A6 in human liver microsomes. Xenobiotica 39, 795–802.
Pfister, C.U., Martoni, A., Zamagni, C., Lelli, G., De, B.F., Souppart, C., et al., 2001. Effect of age and single versus multiple dose pharmacokinetics of letrozole (Femara®) in breast cancer patients. Biopharm. Drug Dispos. 22, 191–197. John Wiley & Sons, Ltd.
Pistilli, B., Paci, A., Ferreira, A.R., Di Meglio, A., Poinsignon, V., Bardet, A., et al., 2020. Serum detection of nonadherence to adjuvant tamoxifen and breast cancer recurrence risk. J. Clin. Oncol. 38 (24), 2762– 2772.
Tanner, J.-A., Tyndale, R.F., 2017. Variation in CYP2A6 activity and personalized medicine. J. Pers. Med. 7 (4), 18.
Waks, A.G., Winer, E.P., 2019. Breast cancer treatment: a review. JAMA 321, 288–300. American Medical Association.
Waterhouse, D.M., Calzone, K.A., Mele, C., Brenner, D.E., 1993. Adherence to oral tamoxifen: a comparison of patient self-report, pill counts, and microelectronic monitoring. J. Clin. Oncol. 11 (6), 1189–1197.
Werk, A.N., Cascorbi, I., 2014. Functional gene variants of CYP3A4. Clin. Pharmacol. Ther. 96, 340–348. John Wiley & Sons, Ltd.